Inflammatory Markers in Leg Ulcer Fluid from Chronic Venous Insufficiency


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Endothelial dysfunction represents an important factor in the pathogenesis of chronic venous insufficiency (CVI) that is a common invalidating disease. The blood stasis that is often associated with CVI leads to ischemia and inflammatory mediators’ secretion, factors that actively contribute to a local inflammatory degradative pattern.

The purpose of our study was to evaluate soluble matrix metalloproteinases (MMPs) and their inhibitor (TIMP1) associated to leg ulcer wound fluid in comparison with the tissue localized ones. We aimed to develop an easy-to-perform protocol for the quantification of soluble MMPs / TIMP and associate the obtained levels with the disease stage and prognosis.

The main substrates of MMPs are type IV collagen and gelatin. The following MMPs are presented: MMP-2 (72 kDa gelatinase, gelatinase-A) MMP-9 (92 kDa gelatinase, gelatinase-B), MMP-1 (interstitial collagenase). Overall, all MMPs are inhibited by TIMPs once they are activated. The balance between activated and inhibited MMPs contributes to the degradative pattern of the tissue.

**Results**

- When detection of MMPs from CVI leg ulcer fluid is aimed, samples need to be used in higher than recommended serum dilution, especially when MMP9 is to be detected;
- Due to the individual protein pattern of the harvested fluids, specific MMP or inhibitor activities need to be related to the total protein concentration of the fluid sample;
- Comparing the values obtained from normal sera (at the same protein concentration) in the CVI fluids MMP9 values are 4 x more elevated, MMP2x and MMP1 19x, while their inhibitor TIMP1 is 200x reduced;
- Higher values of soluble MMPs and lower TIMP1 were directly associated with disease recurrence;
- Various patterns of soluble MMPs and TIMP-1 were associated with the disease progression, but overall higher MMPs value and lower TIMP1 indicated a poor healing score;
- The studied patients did not present any abnormal seric values of MMPs and TIMP1;

**Methods**

- Soluble MMPs detection - Luminex IS 200 System, xMAP technology using Fluorokine_ MAP multiplex kit; Human MMP for the detection of MMPs: 1, 2, 3, 7, 8, 9, 12 and 13.
- Soluble TIMP-1 - ELISA using Quantikine Human TIMP-1 Immunoassay.
- Protein profiling of the samples was performed using LabChip microfluidic technology (BioRad) and Experion automated electrophoresis system. Experion™ Pro260 Analysis Kit was used in this system for protein ranging from 10 to 260 kDa in mass.
- Controls – normal human sera from 25 volunteers with matching ages were used according to the technical specification of each method.

**Conclusion**

The used methodology can be developed for simultaneous detection of multiple soluble inflammatory markers in specific tissue sites.

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**European Biomarkers Summit**

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